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Link-A-Light ATTO425 Conjugation Kit (1 labeling reaction, 1-2 mg)

Catalog No.: AC018-003

Quantity: 1 kit

Reaction: 1 labeling reaction, 1-2 mg

Kit Contents:

AC018-003-M1:	Link-A-Light Mix (Ready to Use)
AC018-003-R1:	Link-A-Light Modifier reagent (Ready to Use)
AC018-003-Q1:	Link-A-Light Quencher reagent (Ready to Use)

Conjugate: ATTO425

Background: The Link-A-Light conjugation kit allows Fluorescent conjugations to set up in *seconds*, simply by adding a solution of the antibody to be labeled to a proprietary lyophilised mixture containing a proprietary activated Fluorescent Ligand (Figure 1).
By circumventing the desalting or dialysis steps that commonly interrupt traditional protein conjugation procedures, Link-A-Light technology can be used to label small quantities of protein (see Principle).

Label: Atto425 is one of a new generation of Fluorescent labels with a coumarin structure. It has a strong absorption at 436nm, high fluorescence at 484nm (extinction coefficient 4.5 x10e4 cm-1M-1) and high quantum yield.

Applications: Labeling

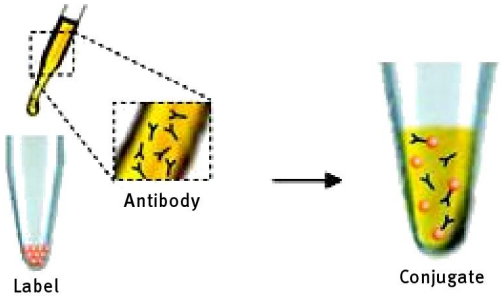


Figure 1

Principle of the Procedure: Upon dissolution of Link-A-Light mixture with a solution of the antibody (or other biomolecule to be labeled) proprietary chemicals in the mixture become activated. This results in coupling of the the antibody to the Fluorescent dye, in a gentle and controlled process at near-neutral pH. Link-A-Light makes it possible to conjugate primary antibodies and other proteins with ease, using a simple, efficient process that has safeguards to prevent over-labeling of the biomolecule and that ensure 100% recovery even at small scale (see Principle).

Frequently asked Questions:

Q1. What functional groups do I need on my protein? Link-A-Light requires amine groups on the molecule to be labeled. Most proteins have lysine and/or alpha-amino groups. All antibodies will have multiple amine functions. **Q2.** Do I need to purify the conjugate? No. The chemicals used in Link-A-Light are deactivated by the Quencher, and the by-products are benign and do not need to be removed. **Q3.** Can Non antibody molecules be labeled? Yes. While labeling of antibodies is a major application, the only requirement is that the protein to be labeled has Amine functionality.

Materials Required but Not Provided:

Antibody to be Labeled: The recommended amount of antibody to be used for labeling is 10-20 µg for **AC018-001**, 100-200 µg for **AC018-002** and 1-2 mg for **AC018-003**. The volume of the antibody sample, ideally, should be in the range 4-10 µl for **AC018-001**, 40-100 µl for **AC018-002** and 400-1000 µl for **AC018-003**. Antibody concentrations of 1-4 mg/ml generally give optimal results, but concentrations and volumes outside the suggested ranges have also yielded excellent conjugates. **Sample Buffer:** Ideally, the antibody to be labeled should be in 10-50mM amine-free buffer pH range 6.5 to 8.5. However, many buffers outside these limits of concentration and pH can be accommodated. Modest concentrations of Tris buffer are also tolerated. **Compatibility of Buffers and Buffer Additives:** Amine-free buffers, including MES, MOPS, HEPES and phosphate are compatible if they are in the concentration range 10-50mM and have pH values in the range 6.5-8.5, as the addition of Link-A-Light Modifier provides the conditions necessary for efficient conjugation. Common non-buffering salts (e.g. sodium chloride), chelating agents (e.g. EDTA) and sugars may be present, as they have no effect on conjugation efficiency. Azide (0.02-0.1%) has little or no effect. If the amine-free buffer is relatively concentrated and outside the pH range 6.6-8.5 you may need to add more Link-A-Light Modifier for each 10 µl of antibody. Excess Link-A-Light Modifier is provided so that you can check the pH of the buffer after the addition of the Modifier. Ideally the pH should be around 7.3-7.6, though efficient conjugation occurs anywhere between pH 6.8 and 7.8. Avoid buffer components that are nucleophilic, as these may react with Link-A-Light chemicals. Primary amines (e.g. amino acids, ethanolamine) and thiols (e.g. mercaptoethanol, DTT) fall within this class. Note: Unusually for an amine, Tris has little effect on conjugation efficiency as long as the concentration is 20mM or less.

Protocols:

1. **Before** you add antibody to the Link-A-Light Mix, add 1 µl of Link-A-Light Modifier reagent for each 10 µl of Antibody to be labeled. Mix gently.
2. Remove the screw cap from the vial of Link-A-Light Mix and pipette the antibody sample (with added Link-A-Light Modifier) directly onto the lyophilised material. Resuspend gently by withdrawing and re-dispensing the liquid once or twice using a pipette.
3. Place the cap back on the vial and leave the vial standing for 3 hours at room temperature (20-25°C). Alternatively, and sometimes more conveniently, conjugations can be set up and left overnight, as the longer incubation time has no negative effect on the conjugate.
4. After incubating for 3 hours (or more), add 1 µl of Link-A-Light Quencher FD reagent for every 10 µl of antibody used. The conjugate can be used after 30 minutes.

Add. Information:

Applicable to Western Blotting, ELISA, Immunohistochemistry, Immunofluorescence, and FACS analysis.

Storage:

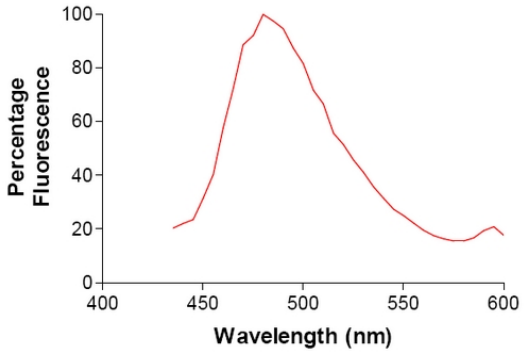
Storage of Conjugates: For any new conjugate, initial storage at 4°C is recommended. A preservative may be desirable for long-term storage. Other storage conditions (e.g. frozen at -70°C or stored at -20°C with 50% glycerol) may also be satisfactory. The best conditions for any particular conjugate must be determined by experimentation.

Storage of Kit: The kit is shipped at ambient temperature in a tamper-evident polypropylene container. Store at -20°C upon receipt.

Pictures:

Absorbance Max (nm)	Emission Max (nm)	Extinction Coefficient (cm^{-1} M^{-1})	Fluorescent Colour	Stokes Shift
436	484	45000	Yellow	48

Emission Scan of Link-A-Light ATTO425 (AC018-002 and AC018-003). Scans perormed in TBS, pH8.



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